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Kix domain specific Immunoglobulin A can protect from adverse lung and cerebral pathology induced by *Plasmodium berghei* ANKA

Anil Kumar, Sapna Deore, Santosh Kumar, Tanuja Bankar, Anil Lotke, Pradeep Parab, Musti Krishnasastry*

National Centre for Cell Science, Ganeshkhind Road, Pune, 411007, India

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ABSTRACT

Plasmodium specific IgA has been detected in serum and breast milk among the endemic population but the role it can play *in vivo* is not clear. In this report, we demonstrate the utility of Malaria specific IgA, elicited by peptide sequences (referred as Mpep3 and Mpep4) of region VI of EBA-175 (PfrVI). Immunization of mice with KLH tagged or untagged peptides of Mpep3, Mpep4 or with PfrVI have resulted in specific IgA response that inhibits the *in vitro* invasion of *Plasmodium falciparum* merozoites. Mice having the IgA specific to Mpep4 have exhibited higher tolerance to *Plasmodium berghei* ANKA parasitemia, exhibited several fold lesser sequestration of infected RBC, lesser damage to microvasculature with no signs of perivascular haemorrhage and lesser lung inflammation in comparison to unimmunized mice. In addition, the immunized mice have B-cell population that secrete the IgA specific to PfrVI. These results suggest that the IgA specific to these malarial antigens can confer significant advantage to hosts and it may also reduce the severity of malaria infection.

1. Introduction

Plasmodium falciparum, which causes fatal and life-threatening complications like cerebral and placental malaria, utilizes several redundant ligands to invade erythrocyte membrane to evade immune surveillance [1]. Among the ligands, EBA-175 is the primary ligand for invasion by merozoites. The EBA-175, comprises several regions *viz*. Regions I-VI, is secreted by microneme onto the surface of merozoites and is shed at or around the point of invasion [2,3]. While the regions I and II have the Duffy Binding Domains, the regions III-V have variable functions and the C-terminal Cysteine rich region VI is followed by a transmembrane domain (TMD) with a cytoplasmic tail (Fig. 1A). It is reported that the region VI of EBA-175 (PfrVI) participates in the formation of tight junction complex with host membrane, dimerization of the EBA-175 and also for trafficking to microneme. Moreover, the PfrVI has sequences that are conserved across the DBL family proteins. For example, it contains eight highly conserved Cys residues (Fig. 1B, marked in bold) and a ROM4 cleavage site needed for shedding of EBA-175 form the junctional complex for completion of the invasion cycle. Shedding of this junctional complex occurs irrespective of EBA-175 being used as a primary ligand or not [3]. However, the nature of immune response elicited by this domain and its *in vivo* role has not been investigated in detail.

Antibody response against region II and EBA-peptide 44 (42 aa within the region V) can block the binding of native EBA-175 to human erythrocytes and also inhibits the merozoite invasion *in vitro* [2,4]. Importantly, antibodies against EBLs and RBLs are elicited in a kinetic manner i.e. initially to EBA175, EBA181, EBA140 (involved in sialic acid-dependent pathway), and later to PfRh2 and PfRh4 (involved in sialic acid -independent pathway) [1]. The success of passive immunization also highlights the role of Immunoglobulin (Ig) based therapies since anti-EBA-175 IgG has been able to block the invasion by ~90% [5]. Further, animal models based on passive immunization with mono specific antibodies (raised against synthetic peptides) or adoptive transfer of B-cells (specific to malarial antigens) have also highlighted the role of adaptive immune response against malaria infection [6–9]. In addition, susceptibility of B-cell-deficient hosts has, once again, underlined the role of antibodies in fighting the infection [9].

Hence, it is curious whether or not antibody response, exclusively directed towards the membrane proximal region of PfrVI, can have any role during the erythrocyte invasion step. It appears that

* Corresponding author. National Centre for Cell Science, Savitribai Phule Pune University, Ganeshkhind Road, Pune, 411007, India.
E-mail address: mvks@nccs.res.in (M. Krishnasastry).

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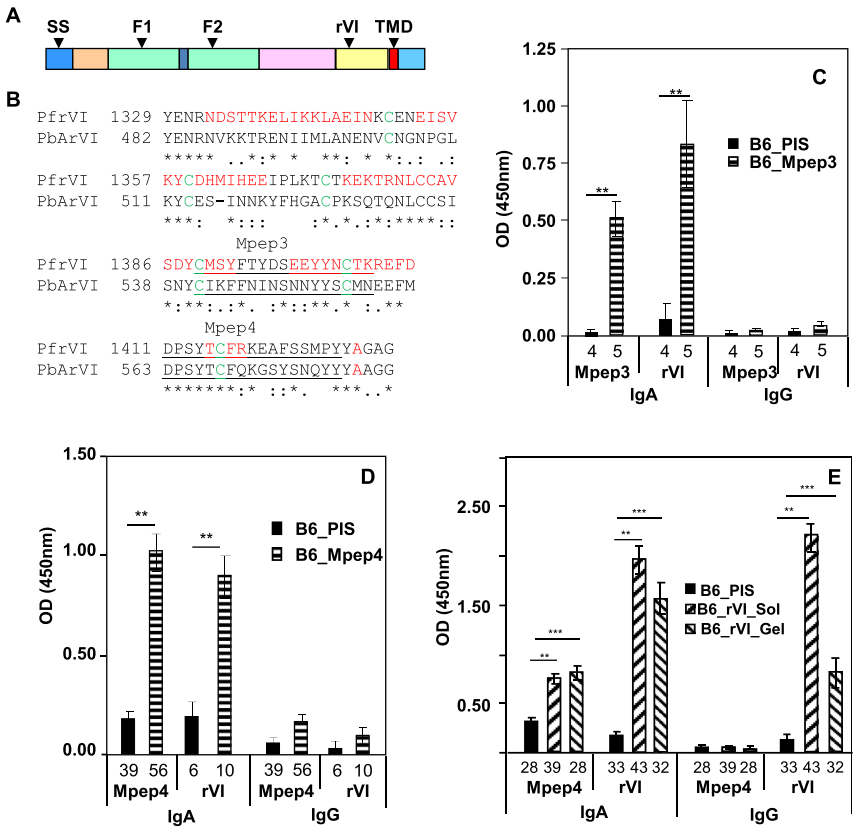

Fig. 1. Immune responses of Mpep3, Mpep4 and PfrVI in mice: **A: Diagrammatic presentation of domains of EBA-175 of *Plasmodium falciparum*.** The markings SS, F1, F2, rVI, TMD respectively represent signal sequence, duffy binding domains 1 and 2, region VI and transmembrane domains. **(B) Homology between PfrVI and PbArVI**: The PfrVI and PbArVI have 44% homology between them and the PfrVI contains a cleavage site (A'G) of PfROM4 protease. The peptides sequences, Mpep3 (17mer) and Mpep4 (18mer), used in the study are underlined. **(C–E)** Immunoglobulin profile elicited by the antigens Mpep3 (C), Mpep4 (D) and PfrVI(E) in C57BL/6 mice: The immunoglobulin isotype was determined by ELISA. The antigen coated on the plate is shown below the group of bars and the isotype detected is shown below for each group. A sample with an OD value which were at least two standard deviations (SD) higher from that of the negative controls (described in methods sections) were considered positive. (**C**) Represents the average data of one of the five independent Mpep3 immunizations of C57BL/6 mice. (**D**) Represents the average data of seven independent immunizations of Mpep4 of C57BL/6 mice. (**E**) Represents the average data of five independent PfrVI immunizations of C57BL/6 mice. The 'sol' and 'gel' form respectively represent the antigen, PfrVI, in solution (purified through Ni-NTA column) and isolated from SDS-PAGE gel specific to the PfrVI band respectively. The number of mice used for obtaining the average for each bar is given below.

the rVI of EBA-175 can play an important role during the erythrocyte invasion step. In this regard, we have observed that the rVI derived peptide sequences i.e. Mpep3 and Mpep4 elicit predominant IgA isotype in Balb/C background. Interestingly, endemic patient sera have also shown positive reactivity towards these sequences. We have also detected the presence of B-cells specific to Mpep3 and Mpep4 in immunized mice and the B-cells secrete IgA in *in vitro* culture (under review else where). In view of these observations, we have attempted to understand the utility of this IgA in the context of experimental animal infection. Since, the Mpep4 sequence has good homology with the rVI domain of *Plasmodium berghei* ANKA (PbA), we have attempted to understand the role of IgA, if any, during the onset of infection. Our results show that the IgA can substantially improve the cerebral pathology of C57BL/6 mice. This can pave the way for understanding the role of IgA in malarial infections.

2. Materials and methods

2.1. Animal experimentation

All protocols were approved by the Institutional Animal Care Committee of National Centre for Cell Science, Pune. All experiments on mice were conducted in pathogen free, approved environment.

2.2. Peptide synthesis

All the peptides used were obtained from commercial sources which were HPLC purified with >99% purity and stored at −80C in dry power form in air tight container. All peptides have been verified by MALDI and MS–MS analysis.

2.3. Cloning of EBA-175 (PfrVI)

The PfrVI construct (coding sequence shown in Fig. 1B), was cloned into pET32a+ using NcoI and HindIII restriction sites and it has 109 amino acid Thioredoxin protein at C-terminus to kill the cytotoxicity associated with PfrVI expression alone. Removal of Trx tag has resulted in precipitation and/or loss of yield. The construct was verified by DNA sequencing.

2.4. Expression and purification of PfrVI by Ni-NTA column

Purification of recombinant PfrVI was essentially carried out using Ni-NTA matrix. *E. coli* BL21(DE3) codon$^+$ strain, transformed